

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the present application.

IN THE CLAIMS:

Claims 1-2. (Canceled).

Claim 3. (Currently Amended) The construct as claimed in claim 34  $\pm$ , wherein the lethal gene is selected from the group consisting of *barnase*, *RnaseTI*, *binase*, *rolB*, *rolC* and a gene encoding diphtheria toxin A.

Claim 4. (Currently Amended) The construct as claimed in claim 34  $\pm$ , wherein the lethal gene is *barnase*.

Claim 5. (Currently Amended) The construct as claimed in claim 34  $\pm$ , wherein the tapetum specific promoter of the first transcription unit is a promoter from a gene selected from the group consisting of TA29, A9, A3, *tap1* and *bcp1*.

Claim 6. (Currently Amended) The construct as claimed in claim 34  $\pm$ , wherein the tapetum specific promoter is from a TA29 gene.

Claim 7. (Currently Amended) The construct as claimed in claim 34 ±, wherein the selectable marker gene is a herbicide resistance-conferring gene selected from the group consisting of *bar*, *ALS*, and *tfdA*, or an antibiotic resistance-conferring gene selected from the group consisting of *nptII*, *hpt* and *aadA*.

Claim 8. (Currently Amended) The construct as claimed in claim 34 ±, wherein the selectable marker gene is *bar*.

Claim 9. (Currently Amended) The construct as claimed in claim 34 ±, wherein the strong constitutive promoter is a CaMV35S promoter.

Claim 10. (Currently Amended) The construct as claimed in claim 34 ±, wherein the insulator sequence comprises coding sequences of a *topoisomerase* gene from pea and an *acetolactate synthase* gene from *Arabidopsis*.

Claims 11-12. (Canceled).

Claim 13. (Currently Amended) A male sterile transgenic plant or seeds thereof, wherein the plant or seeds comprise in their nuclear genome the construct of claim 34 ±.

Claim 14. (Previously Presented) The plant as claimed in claim 13, wherein the plant is selected from the group consisting of a dicotyledonous and a monocotyledonous plant.

Claim 15. (Previously Presented) The plant of claim 13, wherein the plant is *Brassica juncea*.

Claims 16-22. (Canceled).

Claim 23. (Currently Amended) The method as claimed in claim 35 16, wherein ~~the transformed plant cells of step (i) are generated~~ step (A) is carried out by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

Claims 24-33. (Canceled).

Claim 34. (New) An insulator construct for reducing leaky expression of a lethal nucleic acid caused by a strong constitutive promoter, wherein the insulator construct comprises:

(i) a first transcription unit comprising in operable linkage: a tapetum specific promoter, a lethal nucleic acid and a transcription termination sequence, wherein said transcription termination sequence comprises a polyadenylation sequence;

(ii) a second transcription unit comprising in operable linkage: a strong constitutive promoter, a selectable marker

nucleic acid and a transcription termination sequence, wherein said transcription termination sequence comprises a polyadenylation sequence;

(iii) an insulator sequence which is about 5 kb in length, wherein the insulator sequence does not:

- (a) comprise any regulatory or enhancer elements;
- (b) produce a functional RNA or protein; or
- (c) cause homology dependent gene silencing of a host gene;

and wherein the insulator sequence is located between the first transcription unit and the second transcription unit so as to reduce leaky expression of the lethal nucleic acid caused by the strong constitutive promoter in the second transcription unit.

Claim 35. (New) A method of producing male-sterile plants of *Brassica juncea*, wherein said method comprises the steps of:

(A) transforming cells of *Brassica juncea* with an insulator construct comprising:

(i) a first transcription unit comprising in operable linkage: a tapetum specific promoter, a lethal nucleic acid and a transcription termination sequence, wherein said transcription termination sequence comprises a polyadenylation sequence;

(ii) a second transcription unit comprising in operable linkage: a strong constitutive promoter, a selectable marker nucleic acid and a transcription termination sequence, wherein said transcription termination sequence comprises a polyadenylation sequence;

(iii) an insulator sequence which is about 5 kb in length, wherein the insulator sequence does not:

- (a) comprise any regulatory or enhancer elements;
- (b) produce a functional RNA or protein; or
- (c) cause homology dependent gene silencing of a host gene;

and wherein the insulator sequence is located between the first transcription unit and the second transcription unit so as to reduce leaky expression of the lethal nucleic acid caused by the strong constitutive promoter in the second transcription unit;

(B) regenerating transformed plants from the transformed cells;

(C) selecting male-sterile plants from the transformed plants, wherein the male-sterile plants exhibit normal vegetative morphology, normal female fertility, absence of pollen production and failure to set seed on selfing;

(D) selecting from the plants of step (C) male-sterile plants that have a single copy of the insulator construct;

(E) crossing the male-sterile plants from step (D) with untransformed *Brassica juncea* plants to obtain progeny plants; and

(F) selecting from the progeny plants of step (E) plants that exhibit normal seed germination frequencies, normal segregation of the selectable marker nucleic acid, and stable inheritance of male sterility.